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Antioxidant, Anti-Hemolytic and Anti-Inflammatory Activities of Aqueous Extract of Aerial Parts of *Rosmarinus Tournefortii*

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Abstract

Objective: this study aims to evaluate the antioxidant, anti-hemolytic and anti-inflammatory activities of aqueous extract of *Rosmarinus tournefortii* collected from Setif region.

Methods: Total polyphenol and flavonoid contents were determined using Folin-Ciocalteu reagent and aluminium chloride assays, respectively. The *in vitro* antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), β-carotene/linoleic acid and anti-hemolytic assays. The *in vivo* anti-inflammatory activity was assessed in mice using croton oil-induced ear edema and carrageenan-induced paw edema models.

Results: Total phenolic and flavonoid contents of aqueous extract of *R. tournefortii* showed 127.36 ± 7.93 mg Gallic acid equivalent/g of dry weight and 14.75 ± 0.12 mg Quecetin equivalent/g of dry weight, respectively. *In vitro* antioxidant assays revealed that aqueous extract showed important DPPH radical scavenging activity (IC_{50} = 20 ± 0.55 µg/ml) and effective inhibition of peroxidation of linoleic acid of AA % = 61.52 ± 4.00%. The anti-hemolytic test demonstrated that the aqueous extract was more effective than ascorbic acid (used as a standard) in protecting red blood cells. *In vivo* study on anti-inflammatory activity showed that the treatment with aqueous extract of *R. tournefortii* at the doses of 200 and 400 mg/kg possesses a remarkable anti-inflammatory effect in both croton oil- and carrageenan-induced inflammatory models.

Conclusion: Aqueous extract of *R. tournefortii* has potent antioxidant and anti-inflammatory activities and may be important sources of novel antioxidant and anti-inflammatory drugs.

Keywords: *Rosmarinus tournefortii*, antioxidant effect, DPPH, β-carotene, croton oil, carrageenan, inflammation

INTRODUCTION

Inflammation is biological response of the body when its healthy tissues are wounded by physical or chemical stimuli or are invaded by bacteria, viruses, or toxins [1]. This response is generated and maintained by the interactions of inflammatory mediators, such as histamine, kinins, cytokines, eicosanoids, calcitonin gene-related peptide, substance-P, and platelet-activating factor, derived from white blood cells and injured tissues [2]. Inflammation aids in reducing the impacts of potentially harmful microorganisms, removes dead cells that could otherwise cause irritation, and enables damaged tissue to return to its normal state [3].

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Oxidative stress is one of the most common causes of inflammations. It is a phenomenon resulting from an imbalance between production and accumulation of reactive oxygen species (ROS) in cells and tissues and the ability of the biological system to detoxify these reactive products [4]. Oxidative stress can cause various chronic diseases such as cancer, diabetes, neurodegenerative and cardiovascular disease [5].

Plants contain some important types of biologically active compounds, which may supplement the needs of the human body by acting as natural antioxidants. Various studies have shown that plants are rich sources of important phytochemicals such as vitamins A, C, E, and phenolic compounds such as flavonoids, tannins, and lignins [6]. These Phytochemicals could protect the human body from free radicals, prevent oxidative stress and associated diseases.

Rosemary is one of the oldest medicinal plants widely recognized in Algeria. The genus *Rosmarinus* L. (Lamiaceae) is considered as a universal remedy under the Arabic vernacular name *Iklil el djebel* [7]. There are three species of aromatic shrubs native to the Mediterranean Basin that belongs to the Rosmarinus genus, which are: *R. tomentosus* Hub.-Mor. & Maire, *R. officinalis* L. and *R. Tournefortii* de Noé (Syn. *R. eriocalyx* Jord. & Fourr.), and the two last of which grow in Algeria. *R. officinalis* is the best known and the most studied species worldwide, while reports on *R. tournefortii* are limited. These species have been widely utilized in Mediterranean traditional medicine for antiseptic, antispasmodic, stimulating, stomachic and tonic properties [8].

The present study was designed to evaluate *in vitro* antioxidant activity (using DPPH radical, β-carotene and anti-hemolytic assays) and *in vivo* anti-inflammatory activity (using croton oil-induced ear edema and carrageenan-induced paw edema in mice) of aqueous extract of aerial parts of *R. tournefortii*.

MATERIALS AND METHODS

Plant Material

The aerial parts of *Rosmarinus tournefortii* was collected from Ouled Tebben (Setif), Algeria (35° 48′ 46″ north, 5° 06′ 05″ east), during its flowering period, in April 2021. The plant was identified by Prof. Hocine LAOUAR (Laboratory for the Valorization of Natural Biological Resources, Ferhat Abbas University, Setif 1, Algeria) and registered under the N° LVNBR 00435. The plant materiel was dried under shade at room temperature. The dried material was ground to obtain powder which was stored at ambient temperature till use.

Animals

Female NMRI mice (weighing between 25-30 g) were obtained from Pasteur Institute of Algeria, Algiers, and maintained under standard conditions (relative humidity 50-70%, 20–22°C temperature, 12:12 h light: dark cycles). The animals had free access to food and water. The experimental protocol was approved by the Laboratory of Applied Biochemistry, Ferhat Abbas University of Setif 1. All procedures were performed in compliance with laws and institutional guidelines. Animals were kept under laboratory conditions for one week prior to the experiment. This is for their acclimatization.

Preparation of the extracts

Plant extracts were prepared in accordance with Ferreira et al. [9], with slight modifications. The decoction was prepared by boiling 100 g of dried plant material in 1000 ml of distilled water for 20 min. The solution was filtered and dried in an oven at 42°C. The dried extracts

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were kept in the dark at 4°C until tested.

Determination of total polyphenol and flavonoid contents

Total polyphenols in the aqueous extract of *R. tournefortii* were determined using Folin-Ciocalteu reagent, according to Boussoualim et al. [10]. Briefly, 0.5 ml of the Folin-ciocalteu reagent (diluted to 1/10) was added to 0.1 ml of extract or standard (dissolved in methanol or water). After 4 min, 0.4 ml of sodium carbonate were added, and the mixture was incubated for 90 min in the dark. The absorbance was measured spectrophotometrically (thermo spectronic, Helios Epsilon, USA) at 765 nm. The concentration of total polyphenols was expressed as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Total flavonoid content of the aqueous extract was determined using the aluminum chloride colorimetric method. Quercetin was used as a standard. 1 ml of extract (prepared in distilled water) with adequate dilutions, was added to 1 ml of $AlCl_3$ solution (2% in methanol). After incubation at room temperature for 10 min, the absorbance was registered at 430 nm. The concentration of flavonoids was expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW) [10].

DPPH radical scavenging assay

In this method, 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) was used, this free radical is stable at room temperature. Antioxidants reduce the DPPH purple into a yellow product. The measurement of the DPPH radical scavenging activity was performed according to Aouachria et al. [11]. A volume of 50 µl of different concentrations of extract was added to 1250 µl of 0.004 % solution DPPH dissolved in methanol. After 30 min of incubation in dark at room temperature, the absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as standard. The scavenging activity percentage (I %) was calculated using the Eq1:

$$I\ \% = 100 \times (Ac - Ae)/Ac \ldots\ldots \text{(Eq1)}$$

Ac: absorbance of the control,

Ae: absorbance in the presence of extract.

The IC_{50} values (the concentration of the sample that could scavenge 50% of DPPH free radical) were calculated.

β-carotene/linoleic acid bleaching assay

The anti-lipid peroxidation activity of the extract was determined by the linoleic acid/β-carotene system. The method described by Amraoui et al. [12] was used. A stock solution of β-carotene and linoleic acid was prepared with 0.5 mg of β-carotene in 1 ml chloroform, 25 µl of linoleic acid, and 200 mg Tween 40 were added. The chloroform was evaporated by the rotavapor and 100 ml of distilled water saturated with oxygen were then added to the residue. 350 µl of the extract or of BHT were added to 2.5 ml of the above mixture in test tubes. the emulsion system was incubated up to 48 h in dark at room temperature and the absorbance was measured at 490 nm. The antioxidant capacity of the extract was calculated using Eq 2.

$$AA\% = 100 \times (A_t/A_0) \ldots\ldots \text{(Eq 2)},$$

where A_t: absorbance in time t and A_0: absorbance in time 0.

Anti-hemolytic activity

Hemolysis is an indicator of free radical damage affecting the membrane of erythrocytes which might be counteracted by antioxidants. In this assay, 2, 2′,-azobis (2-amidinopropane)

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dihydrochloride (AAPH) was used to generate free radicals, which could attack red blood cells (RBCs) membrane and eventually cause hemolysis. Anti-hemolytic effect of *R. tournefortii* extract was assessed according to the procedure described by Guemmaz et al. [13]. The blood was collected from female mice in tubes containing Etylene Diamine Tetra-Acetic acid (EDTA) and centrifuged for 15 min at 3000 rpm at 4ºC. Supernatant was removed and pellet was washed thrice with Phosphate Bufer Saline (PBS) to obtain a hematocrit of 2%. To induce free radical chain oxidation in erythrocytes, aqueous peroxyl radicals were generated by thermal decomposition of AAPH (dissolved in PBS; 300 mM). To study the protective effects of the *R. tournefortii* extract against AAPH-induced hemolysis, an erythrocyte suspension (2%) was incubated with the extract or standard followed by incubation with AAPH at 37°C for 4 h. The kinetic of hemolysis was monitored by measuring optical density decrease at 630 nm, using a 96-well microplate reader. The resistance of erythrocytes to the radical attack was expressed by the time required to the lyses of 50% of erythrocytes (half-Hemolysis Time, HT_{50} in min). Ascorbic acid (AA) was used as a standard. Negative control contains RBCs and AAPH.

Acute toxicity evaluation

Acute toxicity test was performed according to the organization for economic corporation and development (OECD-425) guidelines [14]. Ethical approval was obtained from the Laboratory of Applied Biochemistry, University of Setif (EAC/LBA 0023/2021). Ten adult mice were fasting overnight with free access to water. The animals were divided into two groups of five animals each. After that, *R. tournefortii* extract was orally administered at a dose of 2000 and 5000 mg/kg body weight. Animals were observed for signs of toxicity, mortalities, changes in general behavior and physical appearance during 14 days. Results of this test permit to determine the doses which will be used in the *in vivo* tests.

Croton oil-induced ear edema in mice

Croton oil-induced mice ear edema was performed according to the method described by Wang et al. [15], with slight modification. Cutaneous inflammation was induced in the inner surface of the right ear of mice by application of 20 µL acetone-distilled water solution (1:1) containing 80µg croton oil as an irritant. The mice were divided into four groups of 5 each as follows: group I: was the vehicle control which received aqueous solution, group II: received Diclofenac (50 mg/kg), and groups III and IV: received 200 and 400 mg/kg of *R. tournefortii* extract, respectively. The animals of all groups were orally treated one hour before the croton oil application. The thickness of the right ear of mice was measured before and 6 h after the induction of inflammation using a digital caliper. The edema was expressed as an increase in the ear thickness due to croton oil application.

Carrageenan-induced paw edema in mice

Carrageenan-induced paw edema is the most commonly used animal model to evaluate the anti-inflammatory potential of pharmacological substances. In this test, four groups of adults mice (n = 5 in each group) received *R. tournefortii* extracts (200, 400 mg/kg), Diclofenac (50 mg/kg) or aqueous solution (vehicle control). The animals were fasted for 12 h before the experiment started with free access to water and treated by oral gavage. After 30 minutes, acute inflammation was induced by injection of 0.02 mL of 1% (w/v) carrageenan in normal saline into the subplantar region of the right hind paw of each mouse [16]. The thickness of the paws was measured using a digital caliper before carrageenan injection and 1, 2, 3, 4, and 5 h after. The percentage of paws swelling was calculated following Eq 3:

$$\% \text{ increase in paw thickness} = 100 \times (D_t - D_0)/D_0 \quad \text{..... (Eq 3),}$$

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where: D_0 is the paw thickness before and D_t is the paw thickness after the carrageenan injection at a given time.

Statistical analysis

The results are expressed as mean ± SD *in vitro* and as mean ± SEM *in vivo*. Statistical analysis of difference was performed using ANOVA, followed by Dunnett test for multiple comparisons, using GraphPad Prism Software (version 7.0). Results were considered statistically significant for $P \leq 0.05$.

RESULTS

Total polyphenol and Total flavonoid contents

The aqueous extract of *R. tournefortii* showed a yield of 9.62%. The total polyphenol content in aqueous extract was 127.36 ± 7.93 mg GAE/g of dry plant, while total flavonoid content was 14.75 ± 0.12 mg QE/g of dry plant.

DPPH radical scavenging effect

The antiradical activity of the aqueous extract was measured by the ability to scavenge DPPH free radicals. The radical scavenging activity was expressed as the mean of the IC_{50} values (concentration needed to give 50% reduction of DPPH radical). The aqueous extract of *R. tournefortii* showed an IC_{50} value of 20 ± 0.55 µg/ml. For comparison, the antioxidant activity is measured by using the same process for BHT used as a standard. The BHT showed an IC_{50} value of 14 ± 0.15 µg/ml.

Linoleic acid/β-carotene bleaching assay

The inhibition of β-carotene oxidation in the presence of *R. tournefortii* aqueous extract and reference antioxidant (BHT), after 24 h of incubation is presented in figure 1.

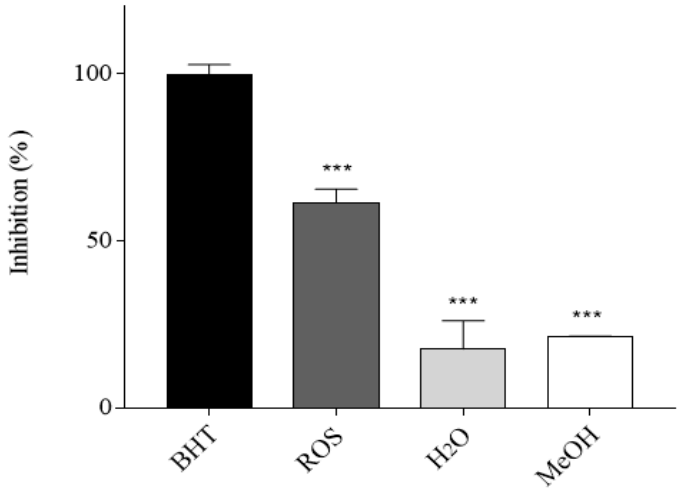

Figure 1: Antioxidant activities of *R. tournefortii* aqueous extract at 24 h of incubation measured by β-carotene bleaching method. ROS: *R. tournefortii*. Values are means ± SD (n = 3). ****p* < 0.001, Comparison was realized against BHT.

In our study, the aqueous extract of *R. tournefortii* exhibited an interesting antioxidant activity (AA %= 61.52 ± 4.00%) compared to BHT (AA %= 99.81 ± 3.04%). The results indicate that

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the extract of the plant exhibits a significant ($p < 0.001$) activity compared to BHT.

Anti-hemolytic activity

The results of Anti-hemolytic activity of plant extracts are shown in figure 2. The results demonstrated that the aqueous extract of *R. tournefortii* exhibited the best protective effect Against AAPH-induced oxidative erythrocytes hemolysis (HT_{50} = 157.34 ± 19.59 min), which was more effective than ascorbic acid (HT_{50} = 73.47 ± 2.10 min). Negative control (AAPH) had an HT_{50} of 60.11 ± 2.55 min.

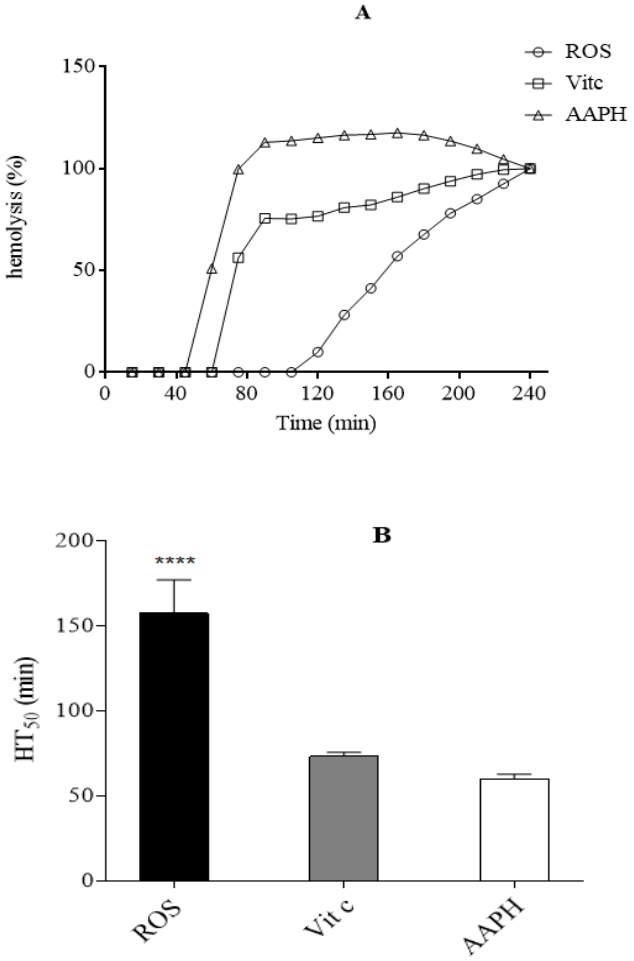

Figure 2: A: Time course of the effect of *R. tournefortii* extract and ascorbic acid on AAPH-induced hemolysis in RBCs. **B:** Half-Hemolysis Time (HT_{50}) for the studied compounds. ROS: *R. tournefortii*, Vit C: vitamin C. Each value represents the mean ± SD (n = 4). ***p <0.001 compared to AAPH as control.

Acute toxicity study

The results of acute oral toxicity test revealed that administration of the aqueous extract of *R. tournefortii* (at 2000 and 5000 mg/kg) did not show any sign or symptom of toxicity in the mice and the surviving mice appears normal and remained alive throughout the 14 days observation. Therefore, it can be concluded that the LD50 value may be greater than 2000 and 5000 mg/kg. As a result, 200 and 400 mg/kg were selected as the doses in order to evaluate *in vivo* anti-inflammatory activity.

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Croton oil-induced ear edema in mice

The effects of orally administered of *R. tournefortii* extract and Diclofenac on croton oil-induced ear edema in mice are shown in figure 3. The mice in the control group that received only the croton oil solution developed after 6 h an edema characterized by an increased thickness (0.124 ± 0.012 mm). The treatment with the aqueous extract of *R. tournefortii* at the doses of 200 and 400 mg/kg induced a very significant reduction ($p < 0.001$) of thickness (0.046 ± 0.004 mm and 0.02 ± 0.005 mm, respectively) compared to the control mice group. In this assay, the standard drug, Diclofenac produced a greater reduction of edema (0.01 ± 0.003 mm).

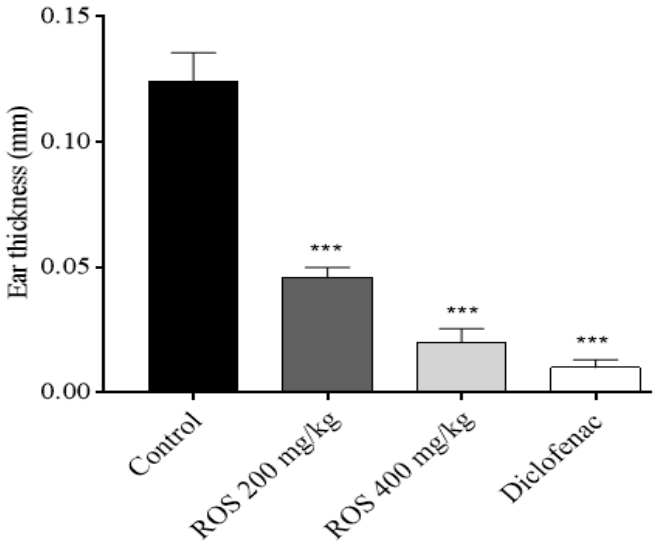

Figure 3: Effect of *R. tournefortii* extract on croton oil-induced ear edema in mice. ROS: *R. tournefortii.* Data are presented as mean ± SEM ($n = 5$). ***$p < 0.001$ when compared with control.

Carrageenan-induced paw edema in mice

Figure 4 show the results of the anti-edematous effect of orally administered of *R. tournefortii* extracts on carrageenan paw oedema in mice. The subplantar injection of carrageenan in the control group caused progressive increase in the paw oedema in the first hour after the injection (32.19 ± 3.79%), presenting the maximum peak at 4h (52.36 ± 2.73%), decreasing to basal level after 5h (47.39 ± 2.40%).

The treatment of animals with Diclofenac caused significant reduction ($p < 0.001$) in paw edema two hours after the subplantar injection of carrageenin (19.18 ± 2.53%), the effect persisting until 5h (12.43 ± 3.38%), compared with control group. The oral administration of *R. tournefortii* extracts at dose 200 mg/kg showed significant inhibitory effects on paw swelling at the interval of 3h ($p < 0.01$) and 5h ($p < 0.001$). Dose of 400 mg/kg promoted significant reduction of edema ($p < 0.001$) at 2h (25.73 ± 5.73%) until 5 h (13.73 ± 3.58%), compared with control group.

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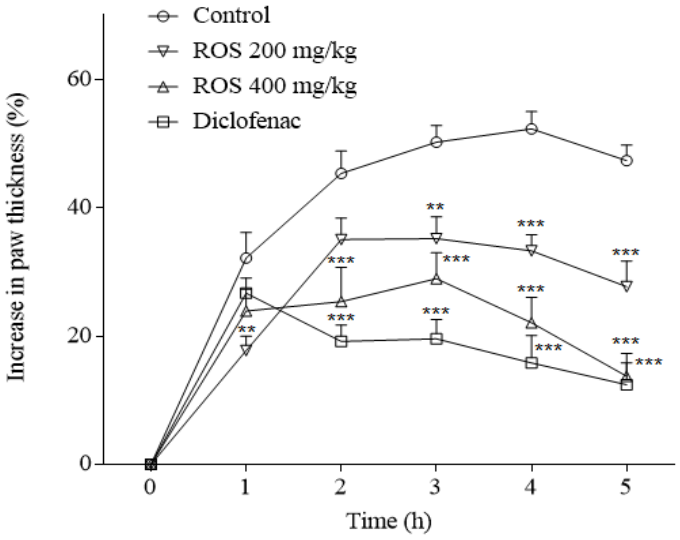

Figure 4: Effect of *R. tournefortii* extract on carrageenan-induced paw edema in mice. ROS: *R. tournefortii*. Data are presented as mean ± SEM (n = 5). **p < 0.01, ***p < 0.001 when compared with control.

DISCUSSION

The use of anti-inflammatory drugs and synthetic antioxidants are associated with a range of serious adverse effects including: gastrointestinal ulcers, cardiovascular risk, liver damage and carcinogenesis [17]. Therefore, the development of alternative anti-inflammatory and antioxidant agents with little or no side effects mainly from natural sources has attracted considerable attention [18]. Medicinal plants are a valuable source of numerous chemicals and/or drugs. In this regard, the purpose of this study was to assess total polyphenol and total flavonoid contents, antioxidant and antihemolytic properties, and anti-inflammatory activity of the aqueous extracts of the aerial parts of *R. tournefortii.*

In the current research work, the amount of total phenolics was determined with the Folin-Ciocalteu reagent. The rate of total phenolics content in the aqueous extract of *R. tournefortii* was estimated at 127.36 ± 7.93 mg GAE/g of dry plant. The total flavonoids content in extract was estimated at 14.75 ± 0.12 mg QE/g of dry plant.

The contents of total phenolics in aqueous extract of *R. tournefortii* are higher than those reported by Bensouici and co-workers [19] in chloroform extract (37.17 ± 7.33 mg GAE/g of dry weight). However, this amount is lower than found in butanolic extract (168.60 ± 3.32 mg GAE/g of dry weight). The same authors reported that the amount of total flavonoids content in chloroform and butanolic extracts (42.43 ± 0.32 mg GAE/g of dry weight and 49.72 ± 1.47 mg GAE/g of dry weight, respectively) were higher than the results of the present study. The variation of phenolics compounds content can be attributed to several factors such as the choice of parts tested, drying and technique of extraction employed, methods of analysis applied, the season of the collection and position of plants, and genotypic differences [20].

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [21]. DPPH is a stable nitrogen-based free radical which has a purple colour. The presence of antioxidant compounds in the medium convert DPPH radical to a more stable DPPH molecular product by donating an electron or a

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hydrogen atom. The reaction is accompanied by colour change from violet of DPPH radical to yellow of reduced form of DPPH measured at 517 nm [22].

The results of DPPH scavenging show that the aqueous extract of *R. tournefortii* exhibited high antioxidant activity (IC_{50}: 20 ± 0.55 µg/ml) which was near to that of BHT (IC_{50}: 14 ± 0.15 mg/ml) used as standard. The IC_{50} of the aqueous extract was higher than the one found by Wafa and Sofiane [23] in aqueous and methanolic extracts (74.62 and 247.21 µg/ml, respectively). The DPPH radical scavenging capacity of extract could be explained by the presence of interesting total polyphenol content [24]. Antioxidant activity of polyphenols is due to their low redox potential and their capacity to donate several electrons or hydrogen atoms [25]. This could play a central role in capturing and neutralizing free radicals in order to prevent their harmful effects.

The β-carotene bleaching assay determines the capacity of an antioxidant to inhibit lipid peroxidation. In this method, a model system made of β-carotene and linoleic acid undergoes a rapid discoloration in the absence of an antioxidant. However, the presence of antioxidant constituents such as polyphenols can reduce the extent of β-carotene destruction by neutralizing the linoleate free radicals and other free radicals formed within the system [26]. The results showed that *R. tournefortii* aqueous extract was able to inhibit lipid peroxidation. It is probable that the polyphenols and flavonoids present in the extract could prevent the bleaching of β-carotene because of their ability to neutralize the free radicals. Numerous studies have indicated that there is a direct relationship between antioxidant activity and polyphenol content of plant extracts [27].

AAPH-induced hemolysis of erythrocytes is a very good model for studying free radical induced oxidative damage to membranes and for evaluating the antioxidant activities of antioxidants [28]. Erythrocytes are particularly susceptible to oxidative stress due to their rich membrane lipid composition of polyunsaturated fatty acids and the high concentrations of oxygen and hemoglobin which promote the oxidative process [29]. Hemolysis was initiated by the peroxyl radicals generated by the decomposition of the water-soluble azo compound (AAPH) at physiological temperature in the presence of oxygen [30]. These radicals attack erythrocyte membranes and induces lipid peroxidation leading to hemolysis. In this study, an assessment was performed on the ability of *R. tournefortii* aqueous extract to protect erythrocytes from oxidative damage caused by AAPH-induced hemolysis. The results indicate that the aqueous extract significantly protected the membrane of the red blood cells from hemolysis better than ascorbic acid, used as a standard. These results demonstrated that the aqueous extract of *R. tournefortii* was able to scavenge the peroxyl radicals derived from AAPH, thereby protecting erythrocyte membrane. The antioxidant activity of the extract can be linked up to the polyphenols and flavonoids content. It has been established a highly positive relationship between polyphenol content and antioxidant activity in plant extracts [31]. The elimination of lipid peroxidation by antioxidants may be due to their free radical scavenging activities. Previously, antihemolytic activity of quercetin and other flavonoids have been reported and good activity of the extracts may be the result of high flavonoid content, especially quercetine [32].

Croton oil-induced ear edema is an *in vivo* model widely used for the investigation of new anti-inflammatory drugs. Croton oil comprises a mixture of lipids, contains 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and other phorbol esters as main irritant agents. In this model, edema events are triggered by protein kinase C (PKC), which activates other enzymatic cascades in turn, such as mitogen activated protein kinases (MAPK) and phospholipase A2 (PLA2), leading to the release of platelet activation factor (PAF) and arachidonic acid. This cascade of events stimulates vascular permeability, vasodilation,

neutrophile migration, release of histamine and serotonin and moderate synthesis of inflammatory eicosanoids by cyclooxygenase and 5-lipoxygenase enzymes [33]. The oral administration of *R. tournefortii* at the doses of 200 and 400 mg/kg reduced significantly the size of the ear edema. These results reveal that the aqueous extract of *R. tournefortii* possesses anti-inflammatory activity against acute inflammation.

The carrageenan-induced paw edema is a model of acute inflammation that a variety of inflammatory mediators are involved in its development, and has been widely used to assess the activity of natural products in resisting the pathological changes associated with acute inflammation [34]. The development of carrageenan-induced paw edema is a biphasic: the first phase is associated with the release of the mediators such as kinins, serotonin and histamine. The second phase is characterised by the infiltration of leukocytes and mediated only by prostaglandins [35]. Oral administration of *R. tournefortii* at the doses of 200 and 400 mg/kg caused a significant reduction of paw edema formation in the second phase. The anti-inflammatory effect of the aqueous extract may possibly be associated with the activities of secondary metabolites. Studies have shown that flavonoids inhibit a number of inflammatory mediators and prevent the synthesis of prostaglandins [36].

CONCLUSION

The results of the present study indicated that the aqueous extract of *R. tournefortii* exhibited significant *in vitro* antioxidant activity and *in vivo* anti-inflammatory effects. Hence, *R. tournefortii* aqueous extract can be used to prevent oxidative and inflammatory processes.

REFERENCES

1. Nistor MT and Rusu AG. Nanorobots with applications in medicine. Polymeric Nanomaterials in Nanotherapeutics. 2019; 123-149.
2. Hussain A, Aslam B, Muhammad F and Faisal MN. *In vitro* Antioxidant Activity and *In vivo* Anti-inflammatory Effect of *Ricinus communis* (L.) and *Withania somnifera* (L.) Hydroalcoholic Extracts in Rats. Braz. arch. biol. technol. 2021; 64: e21200783.
3. Li LS, Chiroma SM, Hashim T, Adam SK, Moklas MAM, Yusuf Z and Rahman SA. Antioxidant and anti-inflammatory properties of *Erythroxylum cuneatum* alkaloid leaf extract. Heliyon. 2020; 6(6), e04141.
4. Abo-Elghiet F, Abd-elsttar A, Metwaly AM and Mohammad AI. Antioxidant, anti-inflammatory, and antimicrobial evaluation of *Terminalia arjuna* leaves, fruits, and bark. Azhar Int J Pharm Med Sci. 2022.
5. Jo NY, Kim KT. Antioxidant Activity and Anti-inflammatory Effect of Extracts from *Paulownia tomentosa* in LPS-stimulated RAW264.7 macrophage cells. J Korean Med. 2019; 40(4):72-83.
6. Gülçin I. Antioxidant activity of food constituents: an overview. Arch Toxicol. 2012; 86: 345-391.
7. Bendif H, Miara MD, Kalboussi Z, Grauzdytė D, Povilaitis D, Venskutonis PR and Maggi F. Supercritical CO2 extraction of *Rosmarinus eriocalyx* growing in Algeria: chemical composition and antioxidant activity of extracts and their solid plant materials. Industrial Crops and Products. 2018; 111, 768-774.
8. Outaleb T, Yekkour A, Hazzit M, Zitouni A and Sabaou N. Phytochemical profiling, antioxidant and antimicrobial effectiveness of *Rosmarinus tournefortii* De Noe extracts issued from different regions of Algeria. Journal of Essential Oil Research. 2020; 32(3), 247-259.

9. Ferreria A, Proenca C and Serralheiro. MLM and Araujo MEM. The *in vitro* screening for acetylcholine esterase inhibition and antioxidant activity of medicinal plant from portugal. Ethnopharmacol. 2006; 108 (1), 31-37.
10. Boussoualim N, Baghiani A, Krache I, Trabsa H, Khennouf S and Arrar L. Inhibitory effects of *Anchusa azurea* extracts on xanthine oxidase activity and its hypouricemic effects on mice. Int J Pharm Pharm Sci. 2015; 7(8), 195-199.
11. Aouachria S, Boumerfeg S, Benslama A, Benbacha F, Guemmez T, Khennouf S, Arrar L and Baghiani A. Acute, sub-acute toxicity and antioxidant activities (*in vitro* and *in vivo*) of *Reichardia picroide* crude extract. J Ethnopharmacol. 2017; 208, 105-116.
12. Amraoui N, Mayouf N, Charef N, Baghiani A and Arrar L. Antioxidant, anti-inflammatory and anti-arthritic activities of methanol extract of *Tamus communis* L. roots. Tropical Journal of Pharmaceutical Research. 2019; 18 (7), 1499-1506.
13. Guemmaz T, Zerargui F, Boumerfeg S, Arrar L, Aouachria S, Khennouf S, Charef N and Baghiani A. Anti-hemolytic, Anti-lipid Peroxidation, Antioxidant Properties and Acute Toxicity of *Xanthium strumarium* Leaves Extracts. Annual Research & Review in Biology. 2018 ; 24(3), 1-12.
14. OECD. Guidelines for the testing of chemicals. Acute oral toxicity: Up and down procedure, 425. 2008.
15. Wang SY, Lan XY, Xiao JH, Yang JC, Kao YT and Chang ST. Antiinflammatory activity of *Lindera erythrocarpa* fruits. Phytother Res. 2008; 22(2), 213-6.
16. Belay R and Makonnen E. Anti-inflammatory activities of ethanol leaves extract and solvent fractions of *Zehneria scabra* (Cucurbitaceae) in rodents. Biofarmasi J Nat Prod Biochem. 2020; 18 (1), 42-56.
17. Bouriche H, Kherbache A, Kada S, Senator A, Demirtas I. Phenolic content, anti-inflammatory and antioxidant activities of *Anacyclus clavatus* extracts. Environmental and Experimental Biology. 2016; 14, 127-135.
18. Yoo T-K, Jeong WT, Kim JG, Ji HS, Ahn M-A, Chung J-W, Lim HB and Hyun TK. UPLC-ESI-Q-TOF-MS-Based Metabolite Profiling, Antioxidant and Anti-Inflammatory Properties of Different Organ Extracts of *Abeliophyllum distichum*. Antioxidants. 2021; 10, 70.
19. Bensouici C, Boudiar T, Kashi I, Bouhedjar K, Boumechhour A, Khatabi L and Larguet H. Chemical characterization, antioxidant, anticholinesterase and alpha-glucosidase potentials of essential oil of *Rosmarinus tournefortii* de noé. Journal of Food Measurement and Characterization. 2019; 14(2), 632-639.
20. Papageorgiou V, Gardeli C, Mallouchos A, Papaioannou M and Komaitis M. Variation of the Chemical Profile and Antioxidant Behavior of *Rosmarinus officinalis* L. and *Salvia fruticosa* Miller Grown in Greece. Journal of Agricultural and Food Chemistry. 2008; 56(16), 7254-7264.
21. Mahmoudi M, Ebrahimzadeh MA, Ansaroudi F, Nabavi SF and Nabavi SM. Antidepressant and antioxidant activities of *Artemisia absinthium* L. at flowering stage. Afr J Biotech. 2009; 8, 7170-7175.
22. Akar Z, Küçük M and Doğan H. A new colorimetric DPPH• scavenging activity method with no need for a spectrophotometer applied on synthetic and natural antioxidants and medicinal herbs. Journal of Enzyme Inhibition and Medicinal Chemistry. 2017; 32(1), 640-647.
23. Wafa N and Sofiane G. Antioxidant, anti-inflammatory and antimicrobial activities of aqueous and methanolic extract of *Rosmarinus eriocalyx* Jord. & Fourr. Int. J. Biol. Chem. Sci. 2020; 14(1): 254-262.
24. Shahidi F, Janitha PK and Wanasundara PD. Phenolic antioxidants. Critical Reviews in Food Science and Nutrition. 1992; 32(1), 67-103.

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25. Zujko ME and Witkowska AM. Antioxidant Potential and Polyphenol Content of Selected Food. International Journal of Food Properties. 2011; 14(2), 300-308.
26. Chaouche TM, Haddouchi F, Ksouri R and Atik-Bekkara F. Evaluation of antioxidant activity of hydromethanolic extracts of some medicinal species from South Algeria. Journal of the Chinese Medical Association. 2014; 77(6), 302-307.
27. Hatami H, Emami SA, Miraghaee SS and Mojarrab M. Total Phenolic Contents and Antioxidant Activities of Different Extracts and Fractions from the Aerial Parts of *Artemisia biennis* Willd. Iran J Pharm Res. 2014; 13(2): 551-559.
28. Takebayashi J, Kaji H, Ichiyama K, Makino K, Gohda E, Yamamoto I and Tai A. Inhibition of free radical-induced erythrocyte hemolysis by 2-O-substituted ascorbic acid derivatives. Free Radical Biology and Medicine. 2007; 43(8), 1156-1164.
29. Jewell SA, Petrov PG and Winlove CP. The effect of oxidative stress on the membrane dipole potential of human red blood cells. Biochimica et Biophysica Acta (BBA) – Biomembranes. (2013); 1828(4), 1250-1258.
30. Banerjee A, Kunwar A, Mishra B and Priyadarsini KI. Concentration dependent antioxidant/pro-oxidant activity of curcumin. Chemico-Biological Interactions. 2008; 174(2), 134-139.
31. Velioglu YS, Mazza G, Gao L and Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agric Food Chem. 1998; 46, 4113-4117.
32. Nabavi SF, Ebrahimzadeh MA, Nabavi SM and Eslami B. Antioxidant activity of flower, stem and leaf extracts of *Ferula gummosa* Boiss. Grasas Y Aceites. 2010; 61 (3), 244-250.
33. Saraiva RA, Araruna MKA, Oliveira RC, Menezes KDP, Leite GO, Kerntopf MR, Costa JGM, Rochac JBT, Tomé AR, Campos AR and Menezes, IRA. Topical anti-inflammatory effect of *Caryocar coriaceum* Wittm. (Caryocaraceae) fruit pulp fixed oil on mice ear edema induced by different irritant agents. Journal of Ethnopharmacology. 2011; 136(3), 504-510.
34. Morales G, Paredes A, Olivares A and Bravo J. Acute oral toxicity and anti-inflammatory activity of hydroalcoholic extract from *Lampaya medicinalis* Phil in rats. Biological Research. 2014; 47, 6.
35. Florentino IF, Nascimento MVM, Galdino PM, Brito AFD, Rocha FFD, Tonussi CR, de Lima TCM, de Paula JR and Costa EA. Evaluation of analgesic and anti-inflammatory activities of *Hydrocotyle umbellata* L., Araliaceae (acariçoba) in mice. Anais Da Academia Brasileira de Ciências. 2013; 85(3), 987-997.
36. Mengie T, Mequanente S, Nigussie D, Legesse B and Makonnen E. Investigation of Wound Healing and Anti-Inflammatory Activities of Solvent Fractions of 80% Methanol Leaf Extract of *Achyranthes aspera* L. (Amaranthaceae) in Rats. J Inflamm Res. 2021; 5(14), 1775-1787.